

Genetic predisposition to wheeze following respiratory syncytial virus bronchiolitis

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Summary

Background The nature of the association between severe respiratory syncytial virus (RSV) bronchiolitis and subsequent wheezing remains unknown. In a previous study, we showed that genetic variation in the IL-8-promoter region is associated with susceptibility to severe bronchiolitis. **Objective** The purpose of this study was to assess the association between wheezing post-bronchiolitis and the genetic variant of IL-8 gene.

Methods We collected data from 134 children who had suffered from bronchiolitis, enrolled in our previous study. The occurrence of wheezing post-bronchiolitis was recorded from a questionnaire sent by post. The association between the genotype and wheezing phenotype was assessed by family-based and case-control approaches.

Results Family-based association showed that the IL-8 variant was transmitted significantly more often than expected in the children who wheezed after the episode of bronchiolitis (transmission = 56%, $P = 0.02$). This effect was not observed in the group of children who had bronchiolitis but did not go on to wheeze. Moreover, the variant was significantly more frequent in post-bronchiolitis wheezers compared with the general population (odds ratio = 1.6, 95% confidence interval 1.0–2.6).

Conclusion These preliminary results suggest that there is a genetic predisposition to wheeze following severe RSV bronchiolitis.

Keywords genetics, IL-8, respiratory syncytial virus bronchiolitis, wheezing

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Introduction

Severe respiratory syncytial virus (RSV) bronchiolitis, independent of atopy, is an important risk factor for recurrent wheezing in childhood [1, 2]. About two-third of infants with severe bronchiolitis will have episodes of wheeze up to the age of 6 years [1]. The nature of the association between RSV bronchiolitis and subsequent wheezing remains unknown. There has been a long-running debate as to whether severe RSV bronchiolitis itself actively promotes the later development of recurrent wheeze, or whether bronchiolitis is just a marker of genetic susceptibility to recurrent airway inflammation [3]. Neutrophil-mediated airway inflammation plays a role in viral-induced wheezing diseases [4]. IL-8 is a potent neutrophil chemokine and activator. We have previously shown that a single nucleotide polymorphism in the promoter region of IL-8 (IL8–251) is significantly associated with severe RSV bronchiolitis [5]. Now we wished to assess whether this variant is also associated with post-bronchiolitis wheezing. If having bronchiolitis directly causes recurrent wheezing, then the genetic variant should be present equally in wheezers and

non-wheezers. If the genetic variant predisposes to wheeze, then it will be found more commonly in wheezers than in non-wheezers. These latter results would support the hypothesis that wheeze and bronchiolitis share a similar genetic background. The occurrence of wheezing after bronchiolitis was retrospectively recorded in the 164 patients enrolled in our previous study.

Patients and methods

The 164 Caucasian children enrolled in our previous study had been admitted to the John Radcliffe Hospital during the first year of life for RSV bronchiolitis, between 1992 and 1998 [5]. DNA was collected and the IL8–251 marker typed as described previously [5]. Between October 2001 and April 2002, we sent a questionnaire to their parents, asking about the occurrence of wheezing. Questions about wheezing occurrence and risk factors were extracted from an existing validated questionnaire [6, 7]. Wheezing was defined as 'breathing that makes a high-pitched whistling or squeaky sound'. The frequency of the IL8–251A allele was assessed within families using the computer program TRANSMIT [8]. This program calculates the transmission of the allele from parents to affected offspring, comparing observed and

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expected frequencies, and can be used when one parental genotype is unknown. The frequency of the genotype of index cases was also compared with that of 706 infants born in the same hospital and determined from cord blood samples. The cord blood samples were collected anonymously. DNA extractions and genotyping of the cord blood samples were carried out at the same time as the patients' samples. Statistical analysis was performed using Stata 7 (Stata Corporation, TX, USA). Categorical variables were compared in both groups using χ^2 and continuous variables using *t*-test. Statistical significance was assigned by a two-sided α level of 0.05. The study was approved by the Central Oxford research ethical committee.

Results

Twelve families could not be contacted, and of the remaining 152 families, 134 parents completed questionnaires (88% of those eligible). Forty-six children (34%) had not wheezed and 88 children (66%) had wheezed after bronchiolitis. Among the 88 children who had wheezed, 42 reported no wheeze over the previous year (defined as transient wheezers); their mean age was 6.7 ± 1.9 years. Forty-six children had had at least one episode of wheezing during the previous year (defined as current wheezers), and their mean age was 6.6 ± 2.2 years. The mean age, gender, number of siblings, history of breastfeeding, nursery attendance, house details, and characteristics of the bronchiolitis (age on admission, oxygen requirement, and duration of hospitalization) were not different in wheezers and non-wheezers (Table 1). Smoking was significantly more common in parents of children who wheezed than in parents of children who did not (odds ratio (OR) = 3.0, 95% confidence interval (CI) 1.1–8.0). Mothers reported suffering from atopic symptoms and from bronchitis (independent of smoking status) more frequently in wheezers than in non-wheezers (OR = 1.7, 95% CI 0.8–3.6 and OR = 3.7, 95% CI 1.0–13.6, respectively) (Table 1).

In 129 families, the genotypes of the child and at least one parent were available. The transmission disequilibrium test (TDT) calculated using TRANSMIT showed that the IL8–251A allele was transmitted more frequently than expected in children who went on to wheeze after bronchiolitis ($P = 0.02$), but not in children who did not wheeze ($P = 0.72$) (Table 2). Parental smoking was the strongest environmental factor associated with wheezing. In the 96 families without this risk factor, the transmission of IL8–251 was further distorted in the 58 families of wheezers but not in those of non-wheezers (Table 2). This observation is consistent with an independent association between the genetic marker and wheezing. The frequency of the A allele in the cord blood samples was 0.46, and the genotypes were in Hardy–Weinberg equilibrium. The proportion of post-bronchiolitis wheezers who carried the A allele (AA homozygotes or AT heterozygotes) was 81% (71/88) compared with 69% (487/706) in the cord blood samples (OR = 1.9, 95% CI 1.1–3.2). The OR associated with homozygote genotype (IL8–251AA) in post-bronchiolitis wheezers was 1.6 (95% CI 1.0–2.6) with reference to the cord blood, and the OR adjusted for age, sex, atopy, and bronchitis in mothers; pre-maturity and smoking of parents was 2.2 (95% CI 0.9–5.7) with reference to post-bronchiolitis non-wheezers.

Table 1. Baseline characteristics of two groups of wheezing

	No wheeze (N = 46)	Wheeze (N = 88)	P-value
Age (years)*	6.9 ± 2	6.6 ± 2.0	0.43
Sex (M/F)	22/24	44/44	0.81
Number of older siblings*	1.3 ± 1.2	1.2 ± 1.0	0.65
Attendance to nursery	17%	22%	0.57
Breastfeeding	66%	53%	0.25
Pets at home	50%	53%	0.71
Pre-maturity	13%	14%	0.92
Age at bronchiolitis (months)*	5.5 ± 5.3	4.9 ± 3.9	0.44
Oxygen during the bronchiolitis	70%	75%	0.53
Duration of hospitalization (days)*	4.7 ± 4.2	4.6 ± 3.8	0.93
Eczema	30%	41%	0.23
Mother with atopy	48%	61%	0.13
Mother with bronchitis	7%	21%	0.04
Parents smoking	13%	31%	0.02

*Mean $\pm$ SD.

When only children without a family history of atopy were considered ($n = 37$), the crude OR for wheezers vs. non-wheezers after bronchiolitis was 8.4 (95% CI 0.77–90.28, $P = 0.04$).

Discussion

This family-based analysis shows excess occurrence of the IL8–251A allele in infants who had RSV bronchiolitis and went on to wheeze. Although there are limitations in parental questionnaires, retrospective recall of wheeze in pre-school period at age 8–13 years has been shown to be a valid proxy measure for the prevalence of wheeze [7]. The similarity between our dataset and those of previous prospective studies suggests that our data accurately represent wheezing symptoms in these children [1, 7]. The association between wheezing and parental smoking replicates previous observations [2]. The children who went on to wheeze did not differ from those who did not, at the time of their admission with bronchiolitis (Table 1). We used family-based analysis because it is more robust than case–control analysis for genetic association studies, due to the difficulty excluding ethnic bias within case–control studies. Population risks cannot be derived from this method and for these estimates we have used 700 cord blood samples collected from the same population to provide a population comparator. Both family-based and case–control analyses support the hypothesis that the excess occurrence of the 251A allele in children who have severe bronchiolitis is accounted for almost entirely by the subgroup of these children who go on to have subsequent wheeze. These children did not have more severe bronchiolitis as assessed by oxygen requirement and length of hospital stay.

Wheezing in early childhood is frequently associated with viral infections [2]. It has been shown that viral-associated wheezing illness is characterized by neutrophil-mediated airway inflammation [9]. IL-8 is a potent neutrophil chemoattractant and activator. The IL-251 variant has not been shown to have a direct effect on IL-8 function. However, this variant has been shown to be associated with a number of

Table 2. Transmission of marker IL-8-251A from parents to affected offspring

Groups (numbers of families)	IL8-251A			χ^2	P-value
	Observed	Expected	% transmitted		
All (129)	140	128.74	54	4.293	0.038
No wheeze (44)	46	44.70	51	0.131	0.717
Wheeze (85)	94	83.97	56	5.557	0.018
Wheeze if non-smoking parents (58)	67	56.72	59	7.791	0.005

The χ^2 and P-values are measures of the significance of the distortion of the observed transmission of the IL8-251A allele from the expected values of 50%.

human diseases [10–13], increasing the likelihood of it being a marker for functional variability. Fine mapping studies of the association between RSV and the IL-8 locus suggest the effect resides on a region of DNA that contains IL-8 and only one other gene, RASSF6, which has no known immunomodulatory function [14]. Functional variation of IL-8 activity remains the most attractive explanation for the disease associations at this locus. Here we show that the association of IL8-251A with severe RSV bronchiolitis is most marked in those children who go on to wheeze. Since viral-associated wheeze appears to be characterized by neutrophilic airway inflammation, this result is consistent with a model in which IL8-251 increases IL-8 production and in this way contributes to increased inflammation. This finding also raises the interesting possibility that this genetic variant is a more general marker of a host response to viral infections that leads to wheezing. If this is so, these children would be at increased risk of viral-associated wheeze whether or not they also had RSV bronchiolitis in infancy. Our preliminary observation of a stronger association between IL8-251AA genotype and wheezing in children without family atopy supports the hypothesis that the pathophysiology of viral-associated wheezing (old-fashioned 'wheezy bronchitis') is different from atopic asthma. It remains to be shown as to whether the association with IL8-251A is specific for wheezing following severe RSV infection or whether it also applies to wheeze independent of bronchiolitis.

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